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SUFFIELD MEMORANDUM

NO. 1291

DETECTION AND IDENTIFICATION OF *VIPERA RUSSELLI* VENOM

by

Shahnaz Siddiqui*, Yunus M. Siddiqui

Jonathon P. Wong and A. Rashid Bhatti

* Term Employee (September 1987-March 1988)

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ABSTRACT

The highly sensitive fluorogenic Enzyme-linked Immunosorbent Assay (FELISA) has been adapted for the detection and identification of *Vipera russelli* venom. The assay sensitivity was observed to be 10^{-13} g ml^{-1}. Venoms from snakes of the *Vipera* group exhibited a high degree of cross reactivity when tested with the antibody raised against *V. russelli* venom. With the exception of venom from *Naja naja*, all the tested venoms from unrelated families also showed cross reactivity. This procedure is useful for detecting snake venom or its components in biological samples.

Key words: snake venom, fluorogenic ELISA (FELISA), nitrocellulose membrane, cross reactivity.

INTRODUCTION

Immunoassays have been extensively used for the identification and quantitation of specific antigens and antibodies in parasitic as well as infectious diseases (1, 2). Attempts have been made to optimize sensitivity without compromising the specificity. This quest has included the search for better solid phase support systems, where the antigen or antibody could be immobilized without losing their concentration or immunoreactivity respectively. Multiple layers of reagents have been used to amplify the sensitivity (2). Antigens have been captured by antibody coated on a solid matrix such as the walls of microtiter plates (3), polystyrene balls (4), dishes (5), silicon rubber (6) and tygon tubing (7) and detection accomplished by enzyme-labelled anti-species antibody and a substrate.

Fluorometric enzyme immunoassays have been found to be more sensitive than photometric assays (8-10). Nitrocellulose membranes have a high binding characterstics for proteins and nucleic acids (11, 12), and their use as a solid phase support in the development of highly sensitive enzyme immunoassays has been reported (13-16). Siddiqui and Fulton (17), have described the long term storage of antibody sensitized on nitrocellulose membranes. These antibody-sensitized membranes were stored at -70°C for over a year and the immunoreactivity of the antibody molecules, as measured by assay sensitivity, was found to be stable.

ELISA has been used to detect the snake venoms and antivenom antibodies. The detection limit of venoms has been reported in the range of 0.5 to 5 ng mL^{-1} (18-20). Although snake venoms are a mixture of complex molecules composed of organic and inorganic moieties, it is the protein components that exert pharmacologic effects upon envenoming

the animal (21, 22). These proteins, either enzymes or polypeptides, are generally toxic and antigenic, but the most antigenic is not necessarily the most toxic (23, 24). These complex molecules may have several antigenic facets which are shared by the venoms of related and non-related snakes (25). Monoclonal antibodies (McA) have been used to map the antigenic determinants present in venoms and Rael *et al.* (26) have reported the cross reactivity among the venoms from the *Crotalinae* snakes. Recently, cross reactivity between non-related snake venoms has been reported (27, 28).

In this communication, we report a procedure that detects 10^{-13} g mL^{-1} of *Vipera russelli* snake venom. Antibody-sensitized nitrocellulose membrane plates, blocked with blocking reagent containing bovine serum albumin and stored frozen at -70°C, showed the same degree of sensitivity as compared with freshly prepared immunoassay plates. The assay was completed within 3 h with the prepared frozen plates whereas assay with freshly prepared plates took 5 h.

MATERIALS AND METHODS

Reagents

All snake venoms, antivenom antibodies against *V. russelli* venom, alkaline phosphatase-labelled anti-horse and anti-rabbit antibodies, diethanolamine (DEA), bovine serum albumin, fraction V (BSA) and the enzyme substrate 4-methylumbelliferyl phosphate (4-MUP) were purchased from Sigma Chemical Co. (St. Louis, Mo). Snake venoms used in this study are listed in Table 1. Phosphate buffered saline (pH 7.4) tablets were from Oxoid Canada, Ltd. (Ottawa, Ontario) and polyoxyethlene sorbital monolaurate (Tween-20) was purchased from Bio-Rad Laboratories (Mississauga, Ontario).

The Millititer™ filtration system and Millititer™-HA immunoassay plates were purchased from Millipore Corporation (Mississuaga, Ontario). The bottom of these plates is made of nitrocellulose membrane of 0.4 um pore size. Each well is separated with a resin glue so there is no possibility of cross contamination of immunoreagents.

Preparation of Reagents

Buffers:

Phosphate buffered saline (PBS), pH 7.4, was prepared according to the manufacturer's instructions.

One liter of 1 M diethanolamine buffer (DEA) was prepared by dissolving 97 mL of DEA in deionized water containing 0.02 M $MgCl_2$ and the pH was adjusted to 9.8 with 1N HCl.

Protein Estimation:

Protein concentrations were determined by the method of Meyer *et al.*, (29) using the bicinchoninic acid (BCA™) protein assay (Pierce) Chemical Company, Rockford, Ill). Crystalline BSA was used as the protein standard.

Enzyme substrate:

4-Methylumbelliferyl phosphate (10^{-4} M) was prepared in DEA buffer immediately prior to use.

Antibody Production and Purification of Immunoglobulins

The antiserum against *V. russelli* snake venom was produced as follows. New Zealand white albino rabbits (Charles River,

St. Constant, P.Q.) were acclimatized for one week in the DRES vivarium from the supplier. They were injected by intramuscular and subcutaneous routes with 1 mL solution containing 1 mg venom adsorbed on 2% sterilized bentonite according to the procedure described elsewhere (30-33). Subsequent injections of 1 mg venom in 1 mL of PBS were given at 2 week interval and the animals were bled 2 weeks following the third injection. Blood was collected, the serum separated and the immunoglobulines were precipitated by the method of Campbell *et al.*, (34). Briefly, saturated solution of ammonium sulfate (pH 8.0) was added to the antiserum in a 1:1 ratio to obtain 50% salt saturation. The solution was stirred and left overnight at 4°C. Precipitated 1gG was collected by centrifugation at 15,000 x g for 15 min in a refrigerated centrifuge (Beckman, J 21-C) and the pellet was suspended in PBS. It was dialysed for 48 h at 4°C against several changes of the same buffer. Horse antiserum against *V. russelli* snake venom was treated in the same manner.

Preparation of Mouse Organ Extracts

Male BALB/c mice (Charles River, St. Constant, P.Q.) were injected with 50 uL of non-adsorbed *V. russelli* venom (100 ug and 1 mg) via three different routes (intramuscular, intraperitoneal and intravenous). They were observed for abnormal signs and symptoms, and later sacrificed by cervical dislocation. Hearts, lungs, livers and brains, along with cardiac blood, were individually collected. Each organ was washed with PBS and weighed. One mL of PBS was added to each organ, in a tissue homogenizer and minced to form a slurry, which was cenrifuged in a refrigerated centrifuge at 35,000 x g for 20 min. Each of the supernates were collected and passed through a 0.45 um filter membrane (Millipore Corp). These samples were tested for the distribution of venom by "sandwich" FELISA procedure (15). Organ preparations from non-venomized mice were used as control.

Standardization of Immunoreagents

Immunochemicals used in the fluorogenic ELISA were titrated against each other to determine the optimum concentration to be used in routine assays (2). The antibody, immobilized on nitrocellulose membrane to capture the antigen, was titrated with the recommended dilution (1:1000) of enzyme-labelled antibody (Sigma Chemical Co.). The concentration of antivenom antibody used as a third layer to amplify the sensitivity was determined by checkerboard titration. Blocking steps were evaluated thoroughly and the experimental conditions that gave the highest ratio between the test and background values were used in this study. Each of the sensitivity and cross-reactivity experiments were repeated several times for their reproducibility.

Immunoassay Procedure

Unless otherwise stated, all incubation for enzyme immunoassay procedures were carried out at 37°C for 1 h. Immune reagents were added to the wells of Millititer^{TM}HA immunoassay plates at a volume of 50 uL. The plates were washed three times with 200 ul of PBS in the Millititer^{TM} filtration system. Horse antivenom-antibody diluted appropriately in coating buffer (Carbonate-bicarbonate pH 9.6 with 0.01% sodium azide), was immobilized on the nitrocellulose support. These plates were incubated overnight at 4°C. Wells were washed twice with PBS to remove excess antibody not attached to the solid phase and the remaining binding sites on nitrocellulose membranes were blocked with 200 ul of blocking buffer (PBS containing 3% BSA and 0.1% Tween-20). The plates were further incubated for 1 h, washed once, and reincubated with blocking buffer for an additional h. Plates were then washed 2 times with PBS and incubated with venom dilutions made in blocking buffer for 1 h. Plates were washed 3 times and rabbit antivenom antibody was added to the wells to amplify the detection of antigen. After

incubation for 1 h and washing with PBS, goat anti-rabbit IgG labelled with alkaline phosphatase was added and the plates were reincubated. Plates were finally washed 6 times with PBS containing 0.05% Tween-20 to remove all unbound enzyme conjugate. After blotting the plate bottom dry, substrate (4-MUP) in a volume of 200 uL was added. Immunoassay plates were incubated at room temperature in the dark and the relative fluorescence was measured in a MicroFLOUR$^{(R)}$reader (Dynatech Laboratories, Alexandria, Va.) at excitation wave length of 365 nm and emission at 450 nm at 5, 10 and 15 min interval.

Data Analysis

Samples (venom dilutions) were tested in six replicate wells and the fluorescence counts from each of the 6 values were averaged and standard deviation calculated. At least 4 separate samples were tested. Background (negative control) values were also averaged and two standard deviations were added to determine the cut-off value. Fluoroscent counts equal to or above this cut-off value defined the lower limit of a positive result.

RESULTS AND DISCUSSION

Optimization of Capture Antibody

Dilutions of horse anti-venom antibodies in coating buffer were titrated with alkaline-phosphatase labelled goat anti-horse IgG. The fluorescence counts suggested that the total saturation of solid phase support with anti-venom antibody was achieved at a concentration of 20 ug mL^{-1}, as the fluorescence counts did not increase with higher anti-venom antibody concentration (Fig. 1).

Sensitivity

V. russelli venom, (10 ug to 1 fg mL^{-1}, diluted in blocking buffer) was used to challenge the "sandwich" format of FELISA. The detection limit for venom was consistently found to be 100 fg mL^{-1}, (Fig. 2). This sensitivity was one log lower than that previously reported from this laboratory for Newcastle disease virus antigen (15) and *Francisella tularensis* outer membrane protein (16). It is well documented that snake venoms are mixtures of complex molecules including both organic and inorganic components. Similar to earlier report (35), we have also found that *V. russelli* venom contained 70 to 80% of protein. Therefore, it was not surprising that detection of venom with FELISA was one log unit less than that of purified Newcastle disease virus antigen and *F. tularensis* outer membrane protein.

It was further observed that the fluorescence counts from the venom concentrations greater than 1 ng mL^{-1} were significantly higher than that reported for Newcastle disease virus antigen (15) and *F. tularensis* OMP (16), thus giving a higher ratio between mean fluorescent counts (MFC) and mean background counts (MBC). Enzymes that hydrolyse paraoxon (diethyl p-nitrophenylphosphate) or similar phosphate substrates have been detected in various snake venoms and *V. russelli* venom has been reported to have significantly higher paraoxonase activity than most other venoms (36-38). Sulkowski *et al.* (39); and Suzuki and Iwanaga, (40) have demonstrated alkaline phosphatase activity in several snake venoms and our study also shows that various levels of similar enzymatic activity were present in different snake venoms. Fifty ul of all 21 snake venoms, at

a concentration of 1 mg mL^{-1}, were titrated with 4-MUP for the detection of alkaline phoshatase activity. The fluorescence counts from each of the venom are presented in Table 2. This suggested that the high MFC/MBC ratio observed was due to the presence of enzymatic activity in venom samples employed.

Specificity and Cross Reactivity

Venoms from 21 snake species belonging to 3 different snake families were tested for their cross reactivity in the FELISA system against *V. russelli* antibody. Standard concentrations of 100 ug and 1 ug mL^{-1} of different venoms were used to challenge the assay procedure while venom from *V. russelli* was used as a positive control. It was consistently observed that, with the exception of venom from *Naja naja*, all the venoms tested showed some degree of cross-reactivity (Fig. 3-5). The venoms from *Vipera* family cross-reacted very strongly, whereas those of *Crotalus* and *Cerastes* showed less cross-reactivity.

The cross reactivity of antigens present in venoms has been studied by a number of investigators (26, 28, 41). Proteins in snake venoms are usually divided in three groups: enzymes, toxins and nerve growth factors (42, 43) and there is a strong possibility that some epitopes of these toxins, enzymes or nerve growth factors, are shared by related or unrelated snakes (25, 26, 44-46). We have also found that the antibodies against *V. russelli* venom reacted not only with the venoms of several members of the same group but also cross reacted with antigens present in venoms of unrealted snakes. The FELISA procedure was unable to identify and recognize the similar epitopes in the native venom of *Naja naja*. Recently, however,

Berger and Bhatti (28) have demonstrated the cross reactivity of *Naja naja* snake venom with the antibodies prepared against *V. russelli* venom by using the Western blot procedure. in their study, they used sodium dodecylsulfate (SDS) to break down venom into polypeptide chains to expose the reactive epitopes.

Detection of Venom in Blood and Tissues

Signs and symptons in mice injected with *V. russelli* venom:

Mice injected with 1 mg *V. russelli* venom by i.p., i.m. and i.v. routes did not survive for more than 30 min. When given the venom via the i.p. route, mice showed excessive urination and became lethargic 5 min after injection. Upon opening the body cavity, it was noticed that there was internal hemorrhage especially in mesenchyma, which was likely due to absorption of venom. Mice injected via the i.m. route exhibited stiffness in hind limbs within 10 min of injection but the front limbs showed no effect. Death of the mice occured in less than 20 min and the eyes turned opaque white. Autopsy revealed the normal morphology of the internal organs but the injection showed severe necrosis and some hemorrhage. The mice injected via the i.v. route died within 5 min. Blood samples collected from these mice showed excessive hemolysis. Visual examination of heart, liver, lungs and brain did not show any abnormality.

In an other set of experiment, two mice each, were injected with a venom concentration of 100 ug via the three routes described above. These mice were observed for 2 h for a change in signs or symptoms, after which they were sacrificed. Mice injected via the i.m. route were lethargic for the first 15 min but appeared normal later. I.p. and i.v. route injections did not cause any visible discomfort to mice

and the autopsy performed on these mice showed no abnormality in internal organs.

Detection of venom

In the experiments where mice were injected with either 1 mg or 100 ug venom via the three different routes, tissue samples were tested for the presence of venom. Organ extracts of a non-venomized mouse were used as negative controls and standard venom concentrations prepared in blocking buffer served as positive controls. Table 3 describes the MFC/MBC ratio obtained in the two experiments.

Irrespective of venom concentration, mice injected via the i.m. route, most of the venom was detected at the injection site. Similar to previous findings (47, 48), blood, heart, liver and brain homogenates showed a trace while more venom was detected in lung preparations. In mice injected via the i.p. route with 1 mg of venom, highest levels of venom were found in liver samples. This may be due to the direct exposure and absorption of venom from the intraperitoneal fluid. Cardiac blood could not be collected from these mice so blood collected from the peritoneal cavity was tested for presence of venom. This blood sample was hemolysed and showed high venom concentration, which was probably due to the contamination of venom present in peritoneal cavity. Mice injected with 100 ug venom showed very little venom in the blood and highest levels were detected in liver preparations. With i.v. injections of 1 mg of venom, the mice died within 5 min. Venom was detected in blood, liver and heart preparations. Traces of venom were also detected in lungs but not in brains. This may be due to the quick death of mice which restricted the venom transport to the brain by blood circulation. One hundred ug of venom injected had no obvious ill effect on the mice and venom was detected in all the organs tested.

It was observed that higher background counts were obtained from the negative controls made of organ extracts from a non-venomized mouse. This discouraged the quantitative estimation of venom present in tissues. The control biological samples were tested for the presence of alkaline phosphatase activity. As depicted in Table 4, brain and liver homogenates were found to have the highest level of alkaline phosphatase activity. Therefore, the high background counts found in the biological samples could be feasible only if the venom standards were prepared in tissue homogenate of the respective organ.

This report describes the detection of *V. russelli* snake venom at a concentration of 100 fg mL^{-1} or 0.5 fg per test volume. This procedure takes only 3 h when pre-prepared immunoassay plates are used to perform the FELISA. Theakston *et al.*, (18) and Coulter *et al.* (20) have described immunoassays for the detection of snake venoms but their assay sensitivity did not exceed 0.5 ng mL^{-1}. The procedure described here is much more sensitive and venom is detected not only in purified form but also in clinical specimens.

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Table 1

VENOMS FROM SNAKES USED IN THIS STUDY THEIR SCIENTIFIC NAME, COMMON NAME AND THEIR DISTRIBUTION

FAMILY VIPERIDAE		
SUBFAMILY VIPERINAE	(MOSTLY FOUND IN AFRICA AND EURASIA)	
VIPERA RUSSELLI	RUSSELL'S VIPER	IRAN TO TAIWAN, INDONESIA.
V. ammodytes	SOUTHERN EUROPEAN SAND VIPER	BALKANS THROUGH TURKEY TO CAUCASUS.
V. palaestinae	PALESTINIAN VIPER	EASTERN MEDITERRANEAN.
V. lebetina	LEVENTINE VIPER	MOROCCO THROUGH ANATOLIA TO KAZAKHSTAN.
ECHIS CARINATUS	SAW-SCALED VIPER	NOTHERN AFRICA TO INDIA AND SRI LANKA
BITIS GABONICA	GABOON VIPER	EQUATORIAL AFRICA TO NATAL
CERASTES CERASTES	DESTER HORNED VIPER	SAHARA-ARABIAN DESERT
ATHERIS SQUAMIGERA	GREEN BUSH VIPER	BELT.

SUBFAMILY CROTALINAE (PIT VIPER)		
AGKISTRODON PISCIVORUS	EASTERN COTTONMOUTH	SOUTHERN USA.
A. rhodostoma	MALAYAN PIT VIPER	INDOCHINA AND ISLANDS.
BOTHROPS JARARACA	SOUTH AMERICAN PIT VIPER	SOUTH AMERICA, SOUTH OF AMAZON BASIN.
B. lansbergii	HOG-NOSED PIT VIPER	CENTRAL AMERICA TO N.W. SOUTH AMERICA.
B. nummifer	JUMPING PIT VIPER	MEXICO TO N.W. SOUTH AMERICA
CROTALUS BASILISCUS	MEXICAN WEST-COAST RATTLESNAKE.	MEXICO.
C.molossus-C.molossus	BLACK-TAILED RATTLESNAKE	NORTHERN MEXICO.
C.viridis oreganus	PACIFIC RATTLESNAKE	WESTERN N. AMERICA.
C. viridis viridis	PRAIRIE RATTLESNAKE	WESTERN N. AMERICA.

Table 1 (Cont'd)

FAMILY ELAPIDAE		
SUBFAMILY ELAPINAE		
NAJA HAJE	EGYPTIAL COBRA	SAHARA INTO ARABIA AND BOTSWANA.
N. melanoleuca	BLACK-LIPPED COBRA	TROPICAL AFRICA.
N. naja	INDIAN COBRA	IRAN TO TAIWAN.
N. najakaouthia	THIALAND COBRA	SOUTH-EAST ASIA.

SOURCE

UNDERWOOD, G. (1978). CLASSIFICATION AND DISTRIBUTION OF VENOMOUS SNAKES IN THE WORLD IN: SNAKE VENOMS. ED. CHEN-YUAN LEE, PP 15 40.

Table 2

DETECTION OF *V. russelli* VENOM IN BIOLOGICAL SAMPLES BY "SANDWICH" FORMAT FELISA. RATIOS OF MEAN FLUORESCENCE COUNT (MFC) FROM THE TEST SAMPLES VERSUS MEAN BACKGROUND COUNT (MBC) FROM CONTROL TISSUES IS PRESENTED. MFC/MBC RATIO ABOVE 1.27 (INCLUDES 2 STANDARD DEVIATIONS) WAS CONSIDERED POSITIVE.

FOOT NOTE:

*SINCE THE BLOOD WAS COLLECTED FROM THE PERITONEAL CAVITY, HIGH CONCENTRATION OF VENOM WAS DETECTED.
@ AVERAGE WEIGHT OF MICE WAS APPROXIMATELY 25 G.
N.D. = NOT DONE

BIOLOGICAL SAMPLES	MFC/MBC RATIO FROM FELISA					
	MICE INJECTED WITH 1 MG VENOM			MICE INJECTED WITH 100 MG VENOM		
	I.M.	I.P.	I.V.	I.M.	I.P.	I.V.
BLOOD	1.49	7.8*	3.87	1.50	1.8	5.5
LIVER	1.23	3.8	1.59	1.54	3.32	2.11
LUNGS	2.93	2.01	3.35	3.95	2.38	2.95
HEART	1.48	1.59	1.79	1.34	1.35	2.52
BRAIN	1.17	1.34	2.77	1.29	0.92	1.95
MUSCLE (SITE OF INJECTION)	6.73	N.D.	N.D.	7.59	N.D.	N.D.

Table 3

DETECTION OF ALKALINE PHOSPHATASE ACTIVITY IN SNAKE VENOMS. 50 uL OF EACH VENOM (1 mg mL ^{1}) WAS CHALLENGED WITH 10^{-4} M OF 4-MUP IN DEA BUFFER. FLUORESCENCE COUNTS ARE THE MEAN SD OF THREE DETERMINATIONS ON A SINGLE PLATE READ IN MicroFLUOR (R) FLUOROMETER AFTER 5 MIN OF INCUBATION AT ROOM TEMPERATURE IN THE DARK. FLUORESCENCE COUNTS OF > 4,000 REPRESENT THE MAXIMUM SENSITIVITY OF THE FLUOROMETER.

SNAKE VENOMS	MFC	SNAKE VENOMS	MFC
1. *A. rhodostoma*	> 4000	12. *C. basiliscus*	> 4000
2. *A. piscivorus*	1289 ± 187	13. *E. carintus*	77 ± 17
3. *B. gabonica*	over	14. *N. melanoleuca*	> 4000
4. *A. squamigera*	109 ± 23	15. *N. haje*	> 4000
5. *B. jararaca*	241 ± 32	16. *N. naja*	> 4000
6. *B. lansbergi*	442 ± 27	17. *N. naja kaouthia*	> 4000
7. *B. nummifer*	1011 ± 32	18. *V. ammodytes*	3791 ± 272
8. *C. cerastes*	3915 ± 127	19. *V. lebetina*	3297 ± 127
9. *C. viridis viridis*	54 ± 17	20. *V. palaestinae*	2389 ± 127
10. *C. viridis organus*	> 4000	21. *V. russelli*	3916 ± 231
11. *C. molossus molossus*	> 4000	22. *Blank*	39 ± 7

Table 4

DETECTION OF ALKALINE PHOSPHATASE ACTIVITY IN TISSUE HOMOGENATES OF NON-VENOMIZED MICE. 50 uL OF HOMOGENATES WERE TESTED WITH 4-MUP AND AFTER INCUBATING PLATES FOR 5 MIN AT ROOM TEMPERATURE IN THE DARK, THE FLUORESCENCE WAS READ IN FLUOROMETER. COUNTS ARE THE MEAN OF OBSERVATIONS IN TRIPLICATE AND ERRORS ARE THE STANDARD DEVIATIONS OF MEAN. BLOCKING BUFFER WAS USED AS THE NEGATIVE CONTROL.

BLOCKING BUFFER	ORGAN HOMOGENATE PREPARATIONS					
	BLOOD	HEART	LIVER	LUNGS	BRAIN	MUSCLE
39 ± 17	608 ± 83	743 ±103	199 ± 68	828 ± 173	1887 ± 210	373 ± 42

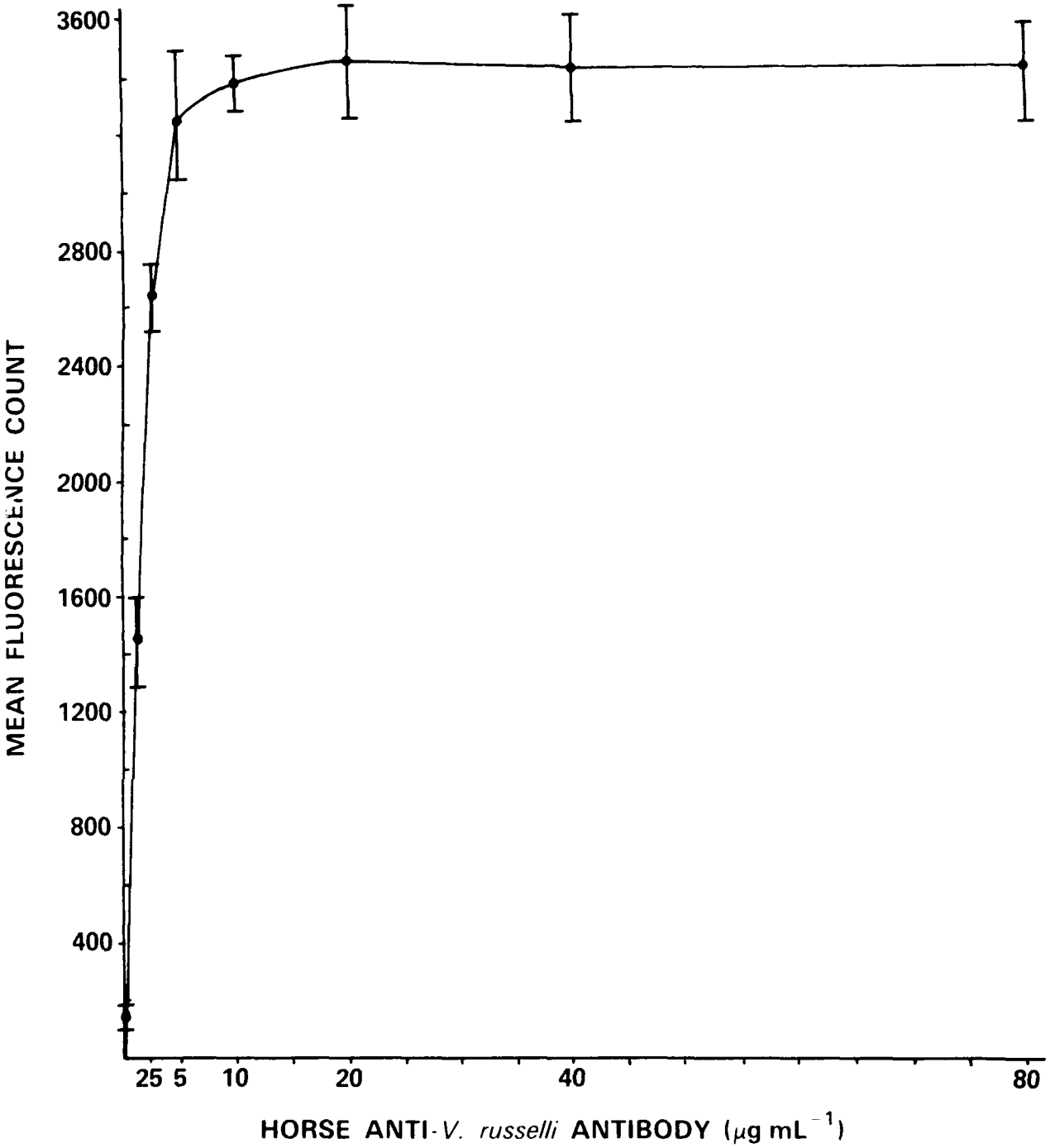

Figure 1

OPTIMIZATION OF CAPTURE ANTIBODY (HORSE ANTI-VENOM ANTIBODY) ON NITROCELLULOSE MEMBRANES. CAPTURE ANTIBODY WAS CHALLENGED WITH ALKALINE PHOSPHATASE-LABELLED ANTI-HORSE IgG. DATA POINTS ARE THE MEAN OF SIX DETERMINATIONS ON SINGLE PLATE. ERROR BARS REPRESENT STANDARD DEVIATION (SD) OF THE MEAN.

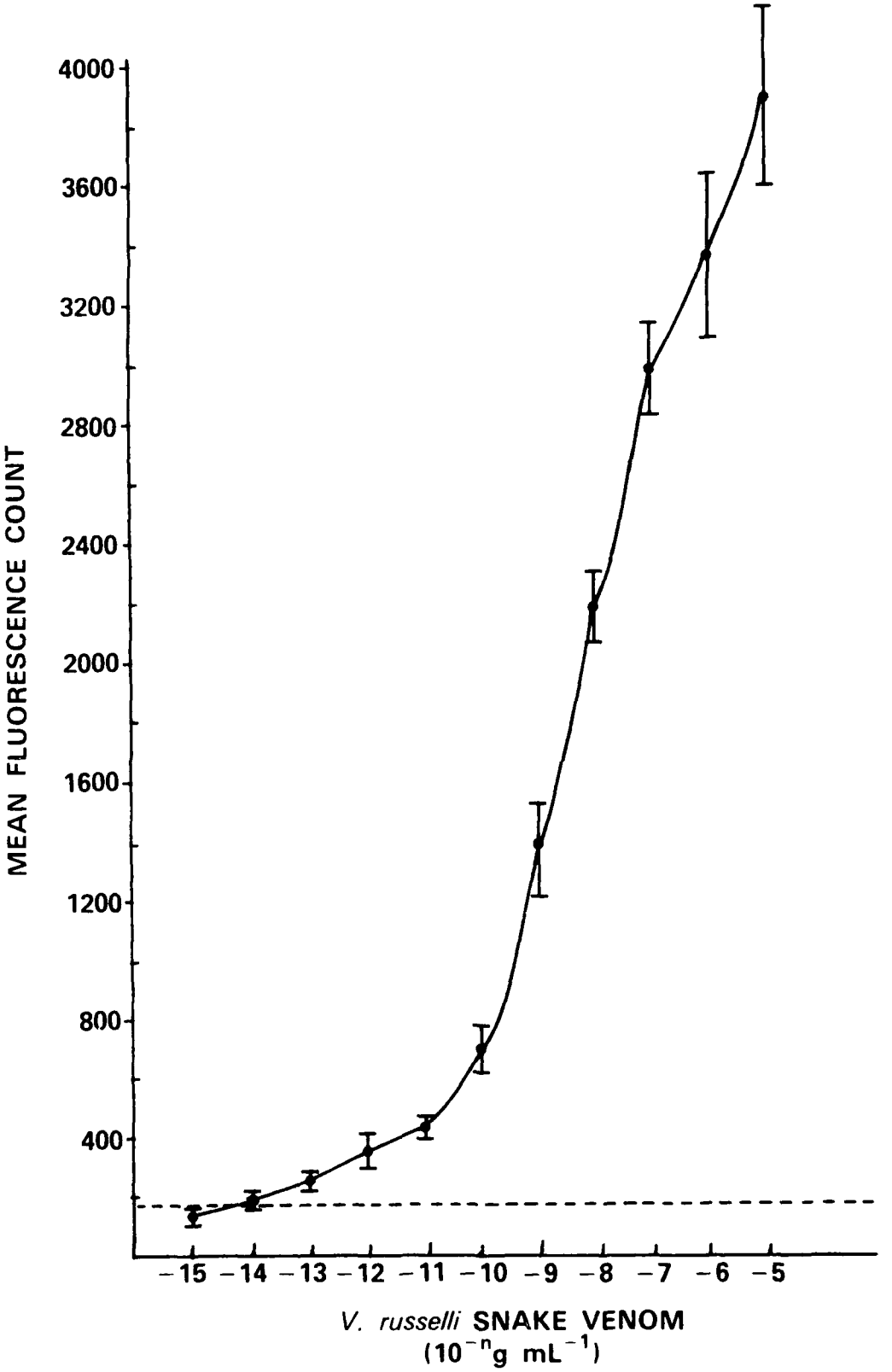

Figure 2

SENSITIVITY OF FELISA FOR THE DETECTION OF *V. russelli* VENOM. DATA POINTS ARE THE MEAN OF SIX DETERMINATIONS OF SINGLE PLATE. ERROR BARS REPRESENT THE SD OF THE MEAN. DOTTED LINE SHOWS THE NEGATIVE CONTROL PLUS TWO SD.

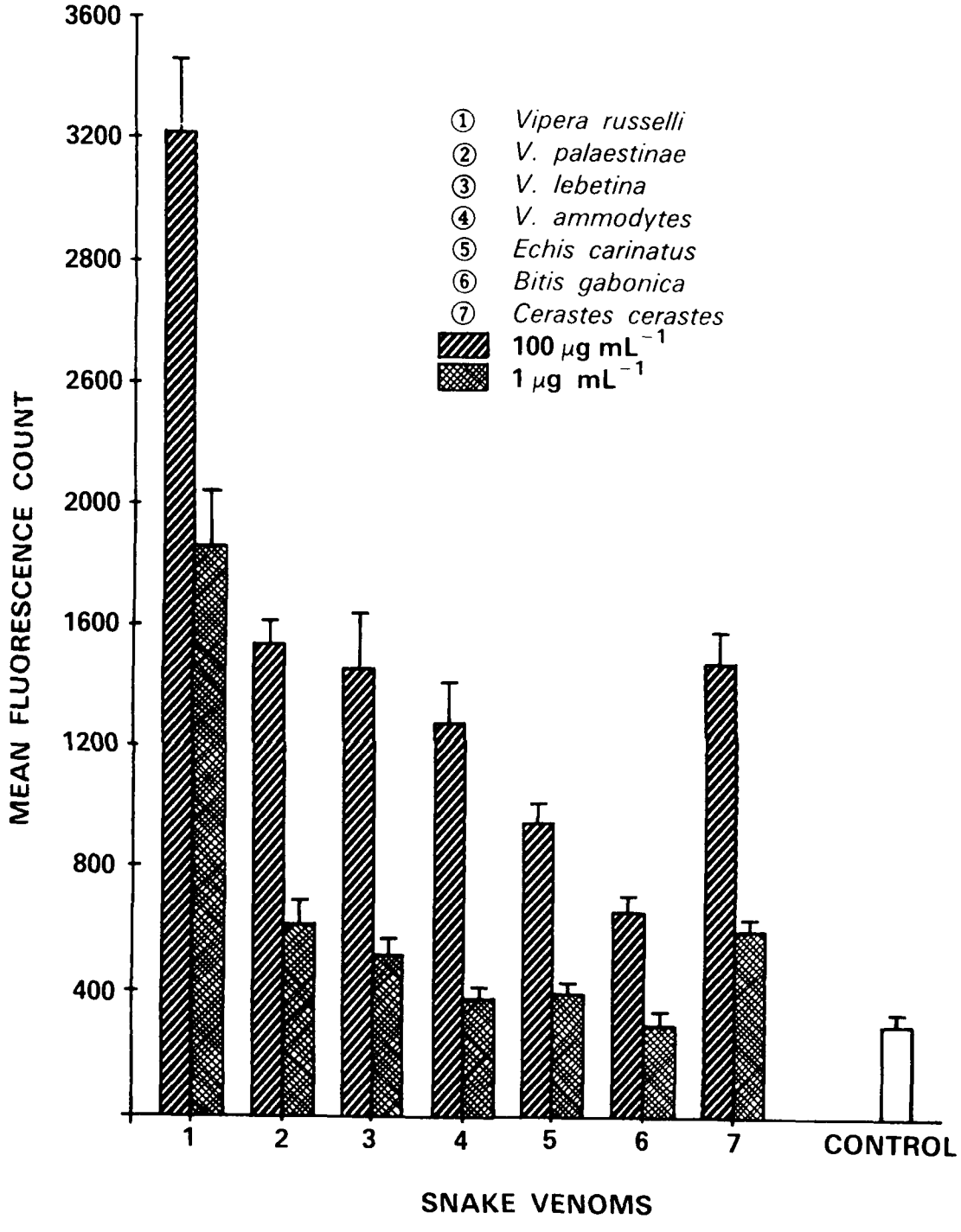

Figure 3

CROSS-REACTIVITY IN SNAKE VENOMS AMONG THE MEMBERS OF FAMILY VIPERIDAE, SUB-FAMILY VIPERINAE. STANDARD CONCENTRATIONS OF 1 AND 100 UG ML^{-1} OF VENOMS WERE USED TO CHALLENGE THE FELISA SYSTEM WITH ANTIBODIES MADE AGAINST *V. russelli* VENOM. BLOCKING BUFFER WAS USED AS NEGATIVE CONTROL. DATA POINTS ARE THE MEAN OF SIX DETERMINATIONS ON SINGLE PLATE AND THE ERROR BARS REPRESENT THE SD OF THE MEAN.

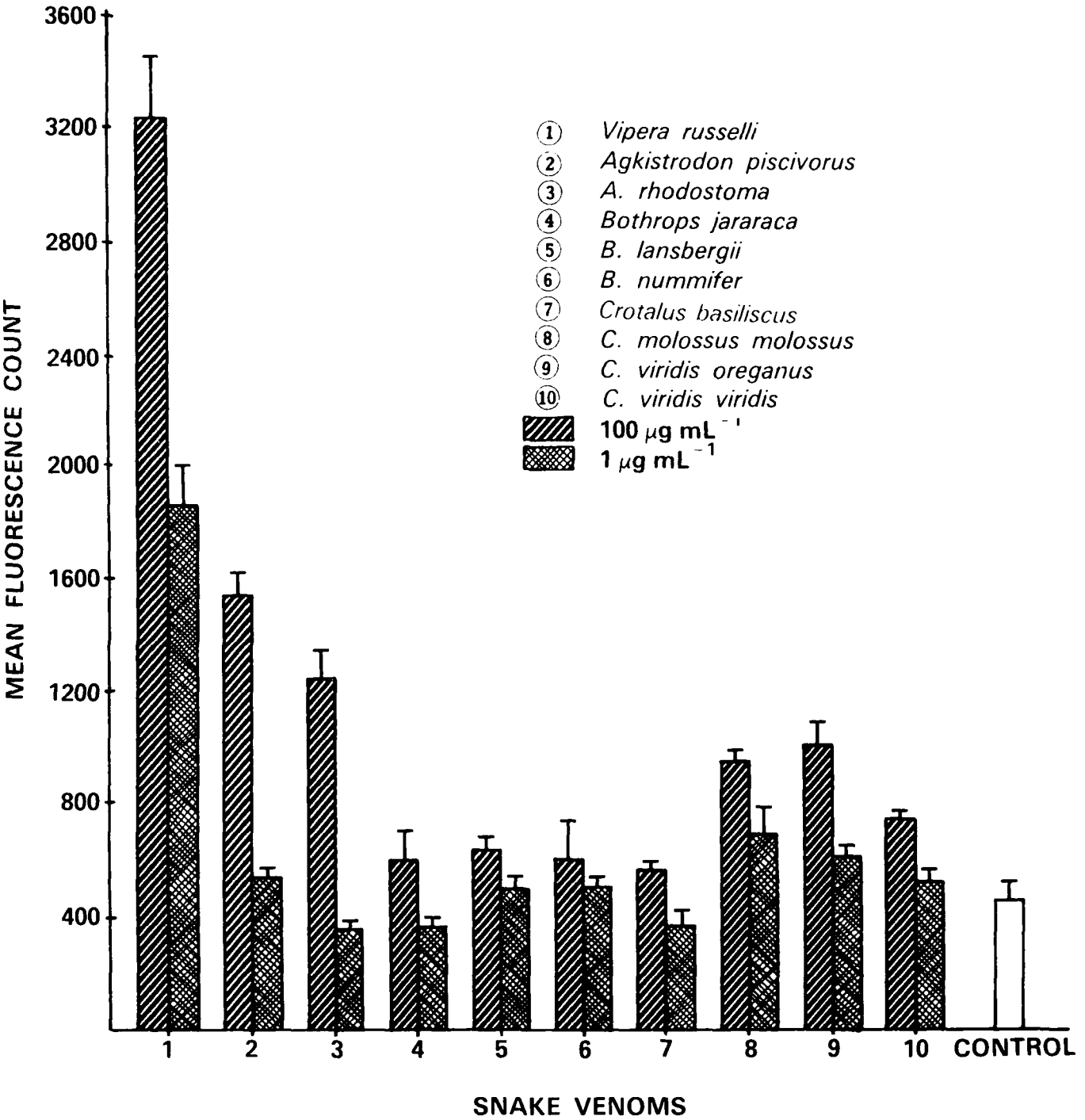

Figure 4

CROSS-REACTIVITY IN SNAKE VENOMS AMONG THE MEMBERS OF FAMILY VIPERIDAE, SUB-FAMILY CROTALINAE. EXPERIMENTAL CONDITIONS WERE AS DESCRIBED IN FIGURE 3.

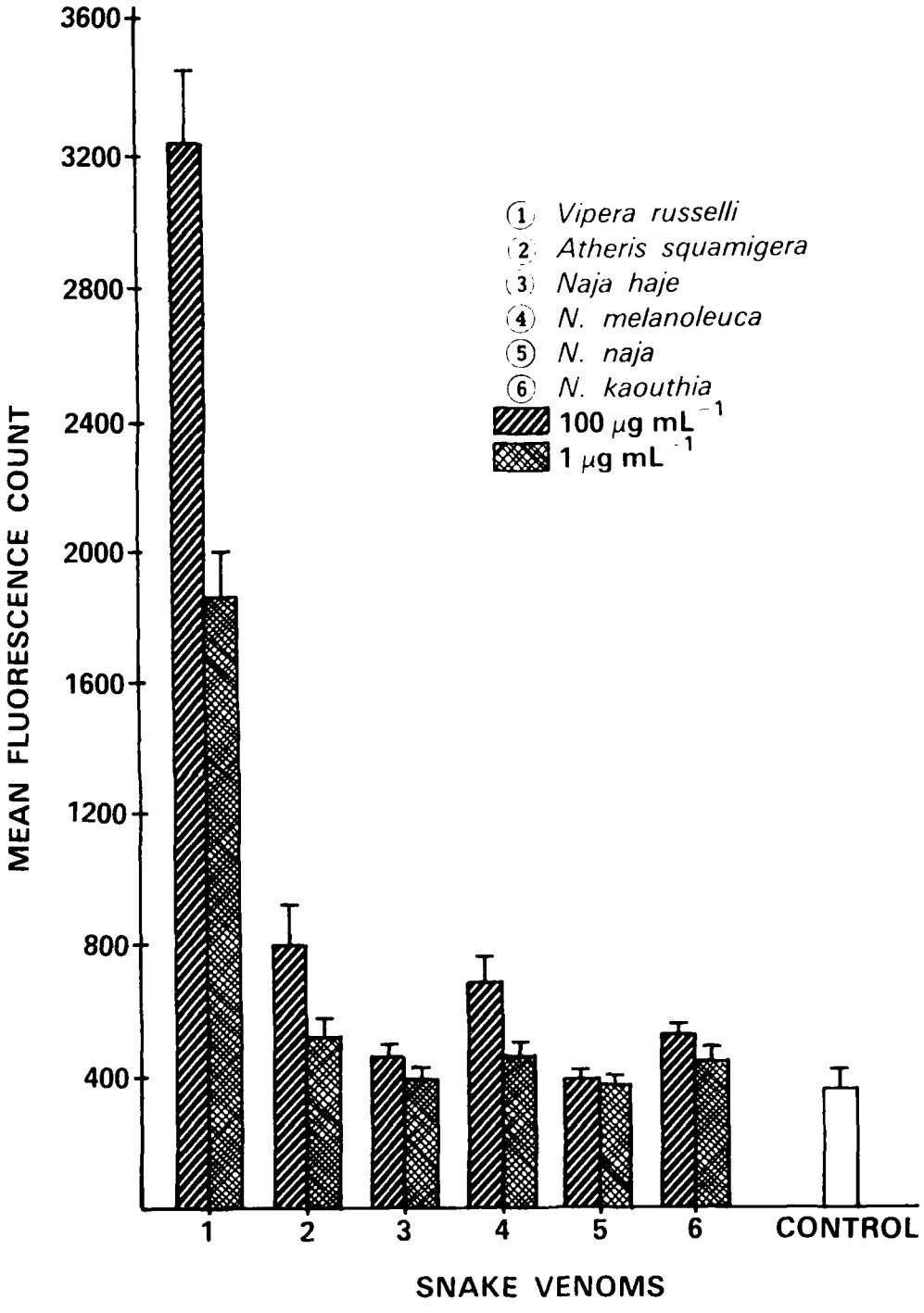

Figure 5

CROSS-REACTIVITY IN SNAKE VENOMS AMONG MEMBERS OF FAMILY ELAPIDAE, SUB-FAMILY ELAPINAE. EXPERIMENTAL CONDITIONS WERE AS DESCRIBED IN FIGURE 3.

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Detection and Identification of Vipera Russelli Venom

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The highly sensitive fluorogenic Enzyme-linked Immunosorbant Assay (FELISA) has been adapted for the detection and identification of Vipera russelli venom. The assay sensitivity was observed to be 10^{-13} g mL^{-1}. Venoms from snakes of the Vipera group exhibited a high degree of cross reactivity when tested with the antibody raised against V. russelli venom. With the exception of venom from Naja naja, all the tested venoms from unrelated families also showed cross reactivity. This procedure is useful for detecting snake venom or its components in biological samples.

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Nitrocellulose Membrane

Cross Reactivity